

We claim:

1. A method for identifying a microorganism that is metabolically
5 similar to a microorganism of interest, comprising:
detecting a difference between the fingerprint spectra of a
microorganism of interest in a first and a second environment; and
comparing the difference between the spectra of the microorganism of
interest in the first and second environments to a difference detected between the
10 fingerprint spectra of a second microorganism in the first and second environments to
determine whether the microorganism of interest responds to a change in environment
similarly to the second microorganism.
2. The method of claim 1 where the first and second environments
15 comprise different growth media.
3. The method of claim 1 where the first and second environments
comprise two batches of the same type of growth medium.
- 20 4. The method of claim 1 where the first and second environments
comprise the same batch of the same growth medium and the environmental conditions
differ in at least one parameter selected from the group consisting of temperature,
humidity, pressure, exposure to light, and exposure to gases.
- 25 6. The method of claim 1 where the first environment is a biological
fluid and the second environment is a growth medium.
7. The method of claim 1 where the first environment is an aerosol and
the second environment is a growth medium.

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8. The method of claim 1 where the fingerprint spectra are selected from the group consisting of mass spectra, electron impact mass spectra, pyrolysis mass spectra, MAB mass spectra, MALDI mass spectra, ESI mass spectra, infrared spectra, Fourier-transform infrared spectra, diffuse reflectance infrared spectra, attenuated total reflectance infrared spectra, ion-mobility spectra, gas chromatograms, fatty-acid methyl ester gas chromatograms, liquid chromatograms, and nuclear magnetic resonance spectra, and portions and combinations thereof.

9. The method of claim 8 where the fingerprint spectra are pyrolysis mass spectra.

10. The method of claim 1 where differences between fingerprint spectra are determined by pattern recognition.

11. The method of claim 10 where pattern recognition comprises statistical pattern recognition.

12. The method of claim 11 where detecting differences comprises determining a vector between two fingerprint spectra represented in canonical variate factor space.

13. The method of claim 11 where detecting differences comprises determining a vector between two fingerprint spectra represented in principal component factor space.

14. The method of claim 1 where comparing differences in the fingerprint spectra of the microorganism of interest and the second microorganism between the first and second environments comprises pattern recognition.

FOOTNOTES

15. The method of claim 14 where pattern recognition comprises statistical pattern recognition.

5 16. The method of claim 15 where comparing differences in the fingerprint spectra of the microorganism of interest and the second microorganism comprises comparing the length and direction of vectors in canonical variate factor space that describe how the fingerprint spectra of the microorganism of interest and the second microorganism change between the first and second environments.

10 17. The method of claim 15 where comparing differences in the fingerprint spectra of the microorganism of interest and the second microorganism comprises comparing the length and direction of vectors in principal component factor space that describe how the fingerprint spectra of the microorganism of interest and the second microorganism change between the first and second environments.

15 18. The method of claim 1 where the method is computer implemented.

20 19. The method of claim 1 where the difference detected between the fingerprint spectra of the microorganism of interest in the first and second environments is compared to differences detected between the fingerprint spectra of two or more microorganisms between the first and second environments to identify the microorganism, from amongst the microorganisms compared, that is most metabolically similar to the microorganism of interest based on a higher degree of similarity between
25 its spectral differences and those of the microorganism of interest than other microorganisms compared.

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20. A method for compensating drift in fingerprint spectra due to changes in environmental factors, comprising:

5 culturing under a first set of environmental factors a microorganism of interest and a second microorganism that is presumably metabolically similar to the microorganism of interest;

measuring a fingerprint spectrum of the microorganism of interest cultured under the first set of environmental factors and a fingerprint spectrum of the second microorganism cultured under the first set of environmental factors;

10 detecting differences between the fingerprint spectrum of the second microorganism cultured under the first set of environmental factors and a fingerprint spectrum of the second microorganism cultured under a second set of environmental factors; and,

15 using the differences between the fingerprint spectra of the second microorganism cultured under the two sets of environmental factors to transform the fingerprint spectrum of the microorganism of interest cultured under the first set of environmental factors to an expected fingerprint spectrum for the microorganism of interest under the second set of environmental factors.

20 21. The method of claim 20 where culturing under a first set of environmental factors comprises culturing on a test growth medium and culturing under the second set of environmental factors comprises culturing on a library growth medium.

25 22. The method of claim 20 where the fingerprint spectra are selected from the group consisting of mass spectra, electron impact mass spectra, pyrolysis mass spectra, MAB mass spectra, MALDI mass spectra, ESI mass spectra, infrared spectra, Fourier-transform infrared spectra, diffuse reflectance infrared spectra, attenuated total reflectance infrared spectra, ion-mobility spectra, gas chromatograms, fatty-acid methyl

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ester gas chromatograms, liquid chromatograms, and nuclear magnetic resonance spectra, and portions and combinations thereof.

23. The method of claim 20 including a step of identifying the
5 microorganism of interest by detecting a similarity between the expected fingerprint spectrum for the microorganism of interest and a fingerprint spectrum of a known organism cultured under the second set of environmental factors.

24. The method of claim 23 where detecting a similarity is
10 accomplished by a pattern recognition method selected from the group consisting of statistical pattern recognition methods, artificial intelligence pattern recognition methods, and combinations thereof.

25. The method of claim 20 where using differences between the
15 fingerprint spectra of the second microorganism cultured under the two sets of environmental factors to transform the fingerprint spectrum of the microorganism of interest to an expected fingerprint spectrum comprises using proportional differences in individual elements of the fingerprint spectra of the second microorganism between the first and second sets of environmental factors to transform the corresponding elements
20 of the fingerprint spectrum of the microorganism of interest cultured under the first set of environmental factors into expected elements of a fingerprint spectrum for the microorganism of interest cultured under the second set of environmental factors.

26. The method of claim 20 where the microorganism of interest is
25 presumed to be a bacterium belonging to a certain class of physiologically similar bacteria and the presumably metabolically similar microorganism belongs to the same class of physiologically similar bacteria.

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27. The method of claim 20 where the microorganism of interest is presumed to be a bacterium belonging to a certain genus of bacteria and the presumably metabolically similar microorganism is of the same genus of bacteria.

5 28. The method of claim 20 where the microorganism of interest is presumed to be a bacterium belonging to a certain species of bacteria and the presumably metabolically similar microorganism is of the same species of bacteria.

10 29. The method of claim 20 where the presumably metabolically similar microorganism is a representative of a metabolic similarity group that exhibits a fingerprint spectrum that is closest in canonical variate or principal component space to the fingerprint spectrum exhibited by the microorganism of interest.

15 30. The method of claim 29 where the presumably metabolically similar microorganism is a distance-weighted composite of two or more representatives of metabolic similarity groups.

20 31. The method of claim 20 where the first and second sets of environmental factors comprise the same batch of the same growth medium and the environmental factors differ in at least one parameter selected from the group consisting of temperature, pressure, exposure to light, and exposure to gases.

25 32. The method of claim 20 where the method is computer implemented.

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33. A method of identifying a microorganism of interest, comprising:
culturing on a test growth medium a microorganism of interest and a
second microorganism that is presumably metabolically similar to the microorganism of
interest;

5 measuring a fingerprint spectrum of the microorganism of interest
cultured on the test growth medium and a fingerprint spectrum of the second
microorganism cultured on the test growth medium;

 detecting differences between the fingerprint spectrum of the second
microorganism cultured on the test growth medium and a fingerprint spectrum of the
10 second microorganism cultured on a library growth medium;

 using differences between the fingerprint spectra of the second
microorganism cultured on the test and library growth media to transform the
fingerprint spectrum of the microorganism of interest to an expected fingerprint
spectrum for the microorganism of interest cultured on the library growth medium; and

15 detecting a similarity between the expected fingerprint spectrum and a
library fingerprint spectrum of a known microorganism.

34. The method of claim 33 wherein the microorganism of interest is a
bacterium and the second, presumably metabolically similar microorganism is a
20 bacterium from a class of bacteria that are physiologically similar to the microorganism
of interest.

35. The method of claim 33 wherein the presumably metabolically
similar microorganism is a bacterium from the same bacterial genus as the
25 microorganism of interest.

36. The method of claim 33, wherein the fingerprint spectra are selected
from the group consisting of mass spectra, electron impact mass spectra, pyrolysis mass
spectra, MAB mass spectra, MALDI mass spectra, infrared spectra, Fourier-transform

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infrared spectra, diffuse reflectance infrared spectra, attenuated total reflectance infrared spectra, ion-mobility spectra, gas chromatograms, fatty-acid methyl ester gas chromatograms, liquid chromatograms, and nuclear magnetic resonance spectra, and portions and combinations thereof.

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37. The method of claim 33, wherein using differences between the fingerprint spectra of the second microorganism cultured on the test and library growth media comprises using proportional differences between individual elements of the fingerprint spectra of the second microorganism between the test and library growth media that, when applied to individual elements of the fingerprint spectrum of the second microorganism cultured on the test growth medium, cause them to resemble corresponding elements of the fingerprint spectrum from the library growth medium.

38. The method of claim 37, wherein using proportional differences between individual elements of each fingerprint spectrum comprises using proportional differences between each element of each fingerprint spectrum.

39. A method for identifying an unknown microorganism, comprising:
measuring a fingerprint spectrum of a microorganism that is presumably metabolically similar to the unknown microorganism and is cultured on a first growth medium;

measuring fingerprint spectra for the unknown microorganism and the presumably metabolically similar microorganism cultured on a second growth medium;

determining a transformation algorithm that converts the fingerprint spectrum of the presumably metabolically similar microorganism cultured on the second growth medium to its fingerprint spectrum when cultured on the first growth medium;

using the transformation algorithm determined for the presumably metabolically similar organism to transform the fingerprint spectrum of the unknown

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microorganism cultured on the second growth medium into an expected fingerprint spectrum for the unknown microorganism cultured on the first growth medium; and, comparing the expected fingerprint spectrum of the unknown microorganism to fingerprint spectra of known microorganisms cultured on the first growth medium to identify the unknown microorganism.

40. The method of claim 39 where determining the transformation algorithm comprises dividing the fingerprint spectrum of the presumably metabolically similar microorganism cultured on the first growth medium by its fingerprint spectrum when cultured on the second growth medium to yield a set of ratios.

41. The method of claim 40 where using the transformation algorithm comprises multiplying the fingerprint spectrum of the unknown microorganism by the set of ratios.

42. The method of claim 39 where comparing the expected fingerprint spectrum of the unknown microorganism comprises comparing to fingerprint spectra in a library database of fingerprint spectra that was assembled using the first growth medium.

43. The method of claim 42 where the comparison is accomplished using computer implemented pattern recognition.

44. The method of claim 39 where the unknown microorganism is a bacterium.

45. The method of claim 44 where the unknown microorganism is a bacterial pathogen and the metabolically similar microorganism is selected from the group consisting of *Salmonella* spp., *E. coli* strains, *Shigella* spp., *Yersinia*

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enterocolitica, *Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp., *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, *Listeria* spp., *Staphylococcus aureus*, *Staphylococcus species*, *Campylobacter jejuni* and other *Campylobacter* spp., and combinations thereof.

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46. The method of claim 39 where the method is computer implemented.

47. The method of claim 39 where the fingerprint spectra are selected from the group consisting of mass spectra, electron impact mass spectra, pyrolysis mass spectra, MAB mass spectra, MALDI mass spectra, ESI mass spectra, infrared spectra, Fourier-transform infrared spectra, diffuse reflectance infrared spectra, attenuated total reflectance infrared spectra, ion-mobility spectra, gas chromatograms, fatty-acid methyl ester gas chromatograms, liquid chromatograms, and nuclear magnetic resonance spectra, and portions and combinations thereof.

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48. A method of assembling a library database of fingerprint spectra for microbial identification, comprising:

measuring a first set of fingerprint spectra for a first set of microorganisms cultured on a library database growth medium;
measuring a second set of fingerprint spectra for a second set of microorganisms cultured on a second growth medium;
detecting differences between the fingerprint spectra of microorganisms that are in both the first and second sets of cultured on the two growth media;
detecting similarities in the differences between the fingerprint spectra of the microorganisms cultured on the two growth media; and
classifying the microorganisms in the library database into metabolic similarity groups based upon the similarities of the differences in their fingerprint spectra.

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FOOTNOTES

49. The method of claim 48 where the second growth medium is a more selective growth medium than the library database growth medium, the second set of fingerprint spectra includes spectra for the microorganisms that will grow on the more selective medium, and the microorganisms that will grow on the second growth medium are classified into metabolic similarity groups.

50. The method of claim 49 where microorganisms to be included in the database that will not grow on the second, more selective growth medium are cultured on a third growth medium that is also more selective than the library database growth medium and the steps of measuring, detecting differences, detecting similarities and classifying are repeated for the subset of microorganisms that will grow on the third growth medium.

51. The method of claim 48 where detecting differences between the spectra includes pattern recognition.

52. The method of claim 51 where pattern recognition is statistical pattern recognition.

53. The method of claim 52 where statistical pattern recognition is principal component analysis or canonical variate analysis.

54. The method of claim 53 where detecting differences includes determining vectors in principal component or canonical variate space between the fingerprint spectra of microorganisms cultured on the two growth media.

55. The method of claim 48 where detecting similarities in the differences between fingerprint spectra includes pattern recognition.

FOOTNOTES

pattern recognition.

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microorganism grown on the test growth medium into a fingerprint spectrum expected for the unknown microorganism if grown on the library database medium.

5 60. The method of claim 59 where representative microorganisms from each of the metabolic similarity groups within the database that will grow on the test culture medium are cultured, their fingerprint spectra are measured, and the representative microorganism that exhibits a fingerprint spectrum most similar to the unknown is used as the representative microorganism for detecting differences.

10 61. The method of claim 59 where representative microorganisms from each of the metabolic similarity groups within the database that will grow on the test culture medium are cultured, their fingerprint spectra are measured, and the spectra of several representative microorganisms that exhibit fingerprint spectra similar to the unknown are combined by distance-weighting to produce a spectrum that is used for
15 detecting differences.

62. A method for identifying a microorganism of interest, comprising:
measuring a fingerprint spectrum of the microorganism of interest in a first environment;
20 transforming the fingerprint spectrum of the microorganism of interest in the first environment to an expected fingerprint spectrum in a second environment using a relationship between the fingerprint spectra of one or more metabolically similar microorganisms between the first and second environments; and
comparing the expected fingerprint spectrum of the microorganism of
25 interest in the second environment to the spectra of known microorganisms in the second environment to identify the microorganism of interest as a known microorganism.

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63. The method of claim 62 where the first environment is a biological fluid.

5 64. The method of claim 62 where the first environment is the atmosphere.

10 65. The method of claim 62 where using a relationship between the fingerprint spectra of one or more metabolically similar microorganisms between the first and second environments comprises using a predetermined, sample-appropriate transform vector.

66. The method of claim 62 where comparing comprises pattern recognition.

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